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INTER-OFFICE CORRESPONDENCE

Richmond, Virginia

To: .Dr. A. C. Lilly Date: February 13, 1990

From: .Dr. G. J. Patskan

Subject: .Operational Plans for the Lowered Biological Activity Program - 1990

I. OBJECTIVE

To decrease the activity of cigarette smoke condensate (CSC) by 90%, relative to 2R1 CSC, as determined by multiple in vitro assays.

II. STRATEGIES

1. Bioassay Development: Establish in vitro bioassays which can differentiate among CSCs from various model cigarettes.

2. Model Development: Prepare model cigarettes designed to reduce biological activity.

3. Model Evaluation: Test CSC from new model cigarettes.

4. Model Optimization: Improve the subjectives of a low activity model.

5. Information Survey: Gather information from the outside scientific community relevant to biological activity.

III. TACTICS AND TIMETABLE

A. BIOASSAY DEVELOPMENT

1. Epidermal Growth Factor (EGF) Binding Assay

a. Status: Arachidonic acid metabolism does not appear to be involved in the reduction of EGF binding caused by CSC. Preliminary experiments investigating the effect of down regulation of protein kinase C on the response to CSC were conducted. The chromatographic removal of catechol from CSC was determined to be too non-selective.

b. Plans:

1. Complete experiments evaluating the effects of down regulation of protein kinase C on the response to CSC (1st Quarter, 1990).

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2. Evaluate the ability of catechol oxidase to degrade catechol in a CSC solution (1st Quarter, 1990).

   a. If the enzymatic degradation of catechol is effective, test the catechol-depleted CSC in the EGF binding assay (2nd Quarter, 1990).

   b. If the enzymatic degradation of catechol is not effective, this work will be discontinued.

3. Determine the effects of CSC on the specific activity of ATP in 3T3 cells in preparation for phosphorylation studies (1st and 2nd Quarters, 1990).

4. Determine the effects of CSC on EGF receptor phosphorylation (2nd and 3rd Quarters, 1990).

2. Phorbol Dibutyrate (PDBu) Binding Assay

   a. Status:

      The assay was not able to distinguish between CSCs following treatment at 37°C or 4°C.

   b. Plans:

      1. The assay will be discontinued and a completion report written (1st Quarter, 1990).

3. JB-6 Transformation Assay

   a. Status:

      TPA, a known tumor promoter, induced the formation of colonies in soft agar.

   b. Plans:

      1. Test the effects of pure compounds which are known to be tumor promoters or are known to be inactive as tumor promoters (1st Quarter, 1990).

      2. Test the response to 2R1 CSC (2nd Quarter, 1990).

      3. a. If 2R1 CSC is active in the standard assay, evaluate the effects of other CSCs (3rd and 4th Quarters, 1990).

      3. b. If 2R1 CSC is not active in the standard assay, explore modifications to the assay which may yield a positive response from 2R1 CSC (3rd Quarter, 1990).

4. Glutathione Depletion Assay (GDA)

a. Status:

Results from experiments with inhibitors of arachidonic acid metabolism were inconclusive.

b. Plans:

1. Determine the relevance of glutathione depletion by CSC in V79 cells using a V79 mutation assay (1st and 2nd Quarters, 1990).

2. a. If the relevance of glutathione depletion is established, then conduct the GDA as a model evaluation tool (3rd and 4th Quarters, 1990).

2. b. If the relevance of glutathione depletion is not established, then direct effort toward other areas of bioassay development (3rd and 4th Quarters, 1990).

B. MODEL DEVELOPMENT

1. Crossed Solubles/Base Web Study

a. Status:

Cigarettes have been prepared from the new feedstocks. A sample of BuCEL low in many components was prepared using electrodialysis. BuCEL was treated with several chelating agents including silver nitrate.

b. Plans:

1. Prepare BuCEL solubles fraction (S1) from new BuCEL and determine the effect of centrifugation conditions (1st Quarter, 1990).

2. Investigate various treatments of BuS1 including: hydrogen peroxide to oxidize amines and irradiation with uv and/or X-rays (1st Quarter, 1990).

3. Evaluate S1 and insolubles fractions from Bu, Br, and Or CELs (1st Quarter, 1990).

4. Complete study of silver nitrate precipitation of components from BuS1 (1st Quarter, 1990).

5. Explore molecular weight separation of BuS1 by hollow fiber ultrafiltration (2nd Quarter, 1990).

6. Consider alternative methods of denitrification for these studies (2nd Quarter, 1990).

7. Provide ninhydrin reactive materials analytical capability (2nd Quarter, 1990).

8. Denitrate BuS1 by electrodialysis (2nd Quarter, 1990).

9. Determine effects of nitrogenous compounds (ammonium salts, standard protein and amino acid mixtures) (2nd Quarter, 1990).

10. Complete study of amino acids and/or sugars (2nd Quarter, 1990).

11. Evaluate the effect of nitrate by adding nitrate to denitrated tobacco solubles (3rd Quarter, 1990).

12. Study precursor/product relationships for S/M activity by addition of appropriate material to electrodialyzed substrate (3rd Quarter, 1990).

13. Evaluate effect of enzyme treatment of BuS1 (4th Quarter, 1990).

14. Complete written report of chemical studies (4th Quarter, 1990).

C. MODEL EVALUATION

1. Salmonella/microsome Assay

a. Status:

CSC from cigarettes prepared with an electrodialyzed sample of BuCEL low in many components on BrBW was low in S/M activity. CSC from cigarettes prepared with silver nitrate-treated BuCEL on BrBW had lower activity than the control.

b. Plans:

1. Substantiate, with more data, the possible change of assay SOP involving storage of CSC dilutions at -80°C (1st Quarter, 1990).

2. Evaluate submitted samples (ongoing).

3. Evaluate samples from Model Development studies (ongoing).

D. MODEL OPTIMIZATION

Laboratory investigations will commence following the development of a model with lowered biological activity.

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IV. RESOURCE ALLOCATIONS (1990)

BIOASSAY DEVELOPMENT:

EGF Assay

D. Stagg (100%)
B. Vaughan (25%)
T. Burruss (20%)
G. Patskan (30%)

PDBu Assay

T. Burruss (10%)
G. Patskan (15%)

JB-6 Transformation

G. Nixon (100%)
T. Burruss (40%)
B. Vaughan (60%)
G. Patskan (20%)

Glutathione Depletion Assay

W. McCoy (100%)
B. Vaughan (15%)
T. Burruss (30%)
G. Patskan (20%)

MODEL DEVELOPMENT:

Crossed Solubles/Base Web Study

S. Hassam (100%)
S. Drew (100%)
R. Hellams (60%)
N. McGee (30%)
R. Izac (100%)
G. Patskan (5%)
R. Kinser (10%)
R. McCuen (10%)

MODEL EVALUATION:

Salmonella/microsome Assay

L. Thompson (100%)
R. Jones (100%)
N. Thompson (10%)
O. Gaines (10%)
G. Patskan (10%)
R. McCuen (10%)

Smoke and Sample Preparation

R. Hellams (30%)
N. McGee (60%)
R. Kinser (5%)

V. LONG RANGE PLANS (1991-1994)

These plans are dependent on: (1) the status on ongoing research; (2) new information, and (3) the availability of resources.

A. BIOASSAY DEVELOPMENT

1991:
1. Develop biochemical assays for JB6 cells.
2. Begin work to develop a mouse "skin" culture system.
3. Establish a protein kinase C assay.

1992:
1. Continue the development of a mouse "skin" culture system.
2. Evaluate possible uses for flow cytometry.

1993:
1. Determine the effects of CSC on the biology of the mouse "skin" culture.
2. Conduct pilot studies using flow cytometry in outside laboratories.

1994:
1. Determine the effects of CSC on the biochemistry of the mouse "skin" culture.
2. Establish flow cytometry capability.

1991-1994: Based on the current information the following topics are also being considered: a cell proliferation assay; an assay based on markers of epithelial differentiation; an intracellular calcium assay; an oncogene assay; a new cell transformation assay; and a free radical assay.

B. MODEL DEVELOPMENT

1991:
1. Evaluate membrane extraction methods.
2. Continue add-back studies.
3. Continue evaluation of precipitation methods.
4. Continue evaluation of alternate methods of denitrification.

5. Continue selective electrodialysis.

6. Identification of precursor components.

7. Evaluate effects of cigarette construction.

1992:

1. Evaluate modified supercritical fluid extraction.

2. Continue evaluation of membrane technology.

3. Evaluate enzyme methods for modification of CEL.

4. Continue selective electrodialysis.

5. Continue identification of precursor components.

6. Continue add-back studies.

7. Evaluate effects of cigarette construction.

1993:

1. Continue add-back studies.

2. Start evaluation of scale-up methods.

3. Continue evaluation of membrane technology.

4. Continue identification of precursor components.

5. Evaluate effects of cigarette construction.

1994:

1. Continue to evaluate scale-up methods.

2. Continue identification of precursor components.

1991-1994: Determine the chemical identity of CSC components responsible for biological activity in new bioassays.

C. MODEL EVALUATION

1991-1994:

1. Continue to evaluate CSC from new model cigarettes using the Salmonella/microsome assay.

2. Evaluate CSC from model cigarettes using new bioassays as they become available.

D. MODEL OPTIMIZATION

Laboratory investigations will commence following the development of a model with lowered biological activity.

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VI. RESOURCE ALLOCATIONS, LONG RANGE (1991-1994)

BIOASSAY DEVELOPMENT:

1991: 3 professionals, 3 technicians
1992: 3 professionals, 3 technicians
1993: 4 professionals, 4 technicians
1994: 4 professionals, 4 technicians

MODEL DEVELOPMENT:

1991: 4 professionals, 1 technician
1992: 4 professionals, 1 technician
1993: 4 professionals, 1 technician
1994: 4 professionals, 1 technician

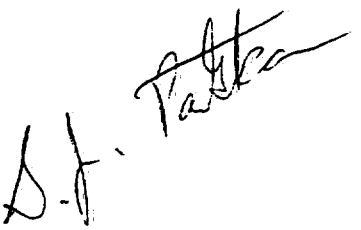
MODEL EVALUATION:

1991: 1 professional, 1.2 technicians
1992: 2 professionals, 2 technicians
1993: 2 professionals, 2 technicians
1994: 2 professionals, 2 technicians

VII. TECHNOLOGY TRANSFER

Selected processes which result in acceptable new model cigarettes will be transferred to the Pilot Plant.

cc: R. Carchman
J. Charles
C. Ellis
O. Gaines
W. Hempfling
R. Jones
R. Kinser
R. McCuen
L. Thompson
N. Thompson
Project 6906
Project 6912



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